Susceptibility of *Maconellicoccus hirsutus* (Homoptera: Pseudococcidae) to Methyl Bromide

J. LARRY ZETTLER,[1] PETER A. FOLLETT,[2] AND RICHARD F. GILL

USDA–ARS, Horticultural Crops Research Laboratory, San Joaquin Valley Agricultural Sciences Center, Parlier, CA 93648

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ABSTRACT Eggs, crawlers, early nymphs, late nymphs, and adults of the pink hibiscus mealybug, *Maconellicoccus hirsutus* (Green), were tested for their susceptibility to methyl bromide in 2-h laboratory fumigations at ambient conditions (25°C, 95% RH). Dose–response tests indicated that the egg was the most susceptible life stage with an LC_{99} of 20.2 mg/liter. Based on probit analysis of dose–response data, no significant differences were observed among susceptibilities of the crawler, early stage or late stage nymphs, or adults at either the LC_{50} or LC_{99} level, but late stage nymphs were more tolerant than early stage nymphs in a separate paired comparison test. Confirmatory tests showed that a dose of 48 mg/liter methyl bromide, the USDA-Animal Plant Health Inspection Service treatment dose schedule for mealybugs at 21–26°C, produced 100% mortality of all life stages. On the basis of these results, we conclude that the methyl bromide treatment schedule for mealybugs will provide quarantine security for *M. hirsutus* infesting commodities for export or import.

KEY WORDS fumigation, quarantine treatment, postharvest, pest control

THE PINK HIBISCUS mealybug, *Maconellicoccus hirsutus* (Green), is a serious economic threat to agriculture, forestry, and the nursery industry. *M. hirsutus* attacks >200 plants, trees, and shrubs including hibiscus, citrus, coffee, sugar cane, plums, mango, pigeon pea, peanut, pumpkin, lettuce, grape, maize, beans, cotton, soybean, and cocoa (Meyerdirk et al. 1998). This mealybug is commonly found throughout tropical and subtropical areas of Africa, India, Egypt, Australia, and Southeast Asia (Williams 1986, 1996; Mani 1989; Reardon et al. 1998). *M. hirsutus* was first detected in the Western Hemisphere in 1983 in Hawaii (Beardsley 1985). *M. hirsutus* was discovered in Grenada in 1994 and has spread rapidly throughout the southern Caribbean region. It is presently found in at least 22 countries and/or islands, including the U.S. Virgin Islands and Puerto Rico, and has spread as far south as the coast of Guyana (Anon. 1998, Kairo 1998, Reardon et al. 1998). *M. hirsutus* is expected to spread quickly to the southeastern United States (Chang and Miller 1996). It was discovered in the cities of Calexico and El Centra, Imperial County, CA, in 1999 (CDFA 1999).

In the Caribbean islands, *M. hirsutus* has become a serious problem attacking many plants, disrupting agricultural trade, and causing significant economic losses. Grenada reported losses of $3.5–10 million for the 1996–1997 season, and Trinidad and Tobago estimate potential losses exceeding $123 million/yr if infestations continue to escalate (Meyerdirk et al. 1998). In California, the mealybug was found in urban areas on fruitless mulberry and fig trees along with trumpet vines and grape vines, but it has not yet been found on agricultural crops. In spite of this, an embargo was initially imposed on interstate shipments of agricultural exports from the infested areas. Currently, international trade has been disrupted only in Mexico and Central America. Mexico has quarantined all imports of host commodities, such as nursery stock, tomatoes, and peppers, originating in Imperial County where *M. hirsutus* has been found. In addition, shipments of table grapes destined for Costa Rica, Guatemala, and El Salvador have been delayed or held up, some requiring fumigation. The economic risk from invasion of *M. hirsutus* into U.S. agriculture has been estimated to be $750 million/yr in the absence of control measures (Sagarra and Peterkin 1999). Because the value of agricultural crops in Imperial County is $1 billion, the presence of *M. hirsutus* poses a significant and immediate economic threat to all of California agriculture.

To cope with quarantine restrictions, fumigation is the preferred option because of its ease of application, quick action, and cost. The existing U.S. Department of Agriculture, Animal Plant Health Inspection Service (APHIS) quarantine treatment schedule for mealybugs is 2-h fumigation with 48 mg/liter of methyl bromide at 21–26°C (64 mg/liter at 16–21°C; USDA 1998). However, no information exists on the effec-

[1] E-mail: lzettler@fresno.ars.usda.gov.

[2] USDA-ARS, U. S. Pacific Basin Agricultural Research Center, Kulani Rd/Stainback Hwy., Hilo, HI.

tiveness of this general methyl bromide treatment against *M. hirsutus.* Thus, our objectives were to determine the effectiveness of methyl bromide against life stages of *M. hirsutus* and to verify the quarantine security of the general USDA-APHIS Treatment Schedule T-104-a-2 for mealybugs.

Materials and Methods

Insects. A colony of *M. hirsutus* was started with nymphs and adults collected from hibiscus plants in Hilo, HI, in 2000. The colony was maintained in the laboratory, first on Japanese pumpkin (*Cucurbita moschata* (Duchesne) var. *chirimen*) using the method described by Meyerdirk et al. (1998), and later on common bean (*Phaseolus vulgaris* L.) in ventilated 3.8-liter plastic tubs (Rubbermaid, Wooster, OH) containing moistened paper towels. Rearing conditions were 25°C, 85% RH, and a photoperiod of 0:24 (L:D). Five life stages were treated: eggs, crawlers, early nymphs, late nymphs, and adult females. During the experiment, eggs were harvested from the colony daily, and all eggs were <2 d old when fumigated. The three nymphal stages were separated by age and size. To establish cohorts of known age, Chinese peas (*Pisum sativum* L.) were placed in emergence tubs containing hatching eggs for 24 h. Peas with young crawlers were removed from tubs daily and transferred to 0.35-liter paper cups with plastic lids (Sweetheart, Chicago, IL) until treatment at a specified age. Fresh peas were provided every 2 d. Crawlers were <2-d old posthatch, early nymphs were 3- to 9-d old, and late nymphs were 10- to 21-d old. Adult females were identified by the presence of an egg mass attached to the dorsum. Treatment of mealybugs on Chinese pea, which is a relatively two-dimensional (flat) host, rather than larger fruit or vegetable hosts, facilitated treatment and counting.

Fumigations. Fumigation chambers (Zettler et al. 2001) were made from wide-mouth Mason jars (0.95 liter). Each jar was made air tight by sealing with a rubber gasketed lid secured tightly with a metal screw ring. The lid was fitted with an injection port consisting of a short length of copper tubing fitted on the outside with rubber tubing that could be closed by pinch clamp. Methyl bromide gas was taken by syringe directly from a commercial compressed gas cylinder (Matheson Tri-gas, Newark, CA) and, depending on the intended treatment dose, injected directly into either the fumigation jar or a 3.8-liter dose jar, similarly fitted with an injection port, for dilution and subsequent dosing.

Before dosing the treatment jar, a volume of air equivalent to 1.5 times the dose volume was removed. After dosing, the partial pressure in the treatment jars was allowed to equilibrate to atmospheric pressure, and the injection port was closed. Methyl bromide concentrations were monitored with a gas chromatograph (GC8A, Shimadzu Scientific, Columbia MD) equipped with a 1-ml gas sample loop, TCD detector, and a 76.2 cm × 0.32 cm Chromasil 330 column (Supelco, Bellefonte, PA) after the method of Obenland et al. (1998). Oven and detector temperatures were 150 and 170°C, respectively; and helium flow was maintained through the column at 20 ml/min. Methyl bromide concentration was calculated from a standard curve. Gas concentration readings were made on each treatment jar at the beginning and end of each exposure to verify actual doses achieved (Zettler et al. 1997).

All treatments were conducted at the USDA–ARS, U.S. Pacific Basin Agricultural Research Center, Hilo, HI. For treatment, *M. hirsutus* life stages on peas (eggs were treated as egg masses attached to females) were placed in 50-ml plastic centrifuge tubes (Nalgene), and tubes with mealybugs were placed in a dose jar. To obtain dose–response data, life stages were exposed to at least five methyl bromide concentrations ranging from 8 to 64 mg/liter and replicated 4–9 times during 2-h fumigations at ambient conditions (25°C, 95% RH). The number of individuals exposed per replicate were: 145–148 adults; 1,051–17,143 crawlers; 730–1,441 early nymphs; 502–3,888 late nymphs; 2,059–4,148 eggs. In addition, a second series of tests consisted of confirmatory treatments using the APHIS treatment schedule's target doses of 48 and 64 mg/liter. When possible, multiple life stages were tested in the same treatment jar, although, in many cases, only one or two life stages could be tested at the same time.

Following treatment, centrifuge tubes with *M. hirsutus* were removed from treatment jars, allowed to aerate under a fume hood for at least 30 min, and held until insects could be counted. Eggs were separated from egg masses, transferred onto double-sided sticky tape (Scotch brand, 3 M, St. Paul, MN) attached to the bottom of a petri dish, and held for emergence (typically, 7 d). Crawlers that emerged from eggs immediately stuck to the tape and were counted easily. Nymphs and adults were held in 0.35-liter paper cups with lids. Nymphs and adults were scored for mortality after 24 h by touching the thorax and abdomen with a camel-hair brush to elicit any leg movement.

Statistical Analysis. Dose–response data, pooled among the replicates, were analyzed by SPSS Professional Statistics to construct dose–mortality regression lines (SPSS 1997). The dose–mortality responses were analyzed in SPSS as individual replicates rather than as summations of all replicates within a given concentration, resulting in large values for chi-square and associated degrees of freedom. Because the values for chi-square were greater than the appropriate tabular values, the analysis used the heterogeneity factor (chi-square divided by degrees of freedom) to correct the standard errors of the parameter estimates (Robertson and Preisler 1992). Regression data were subjected to a likelihood ratio test of equality (Robertson and Preisler 1992) to determine whether the slopes and intercepts of each line were the same (test of parallelism) (SPSS 1997). This test estimates a common slope for all regression lines and compares it separately with each line. In addition, comparative dose–response data for early and late nymphs were subjected to a paired *t*-test (SPSS 1997).

Table 1. Probit regression responses of mortality of five life stages of *Maconellicoccus hirsutus* resulting from 2-h laboratory fumigations with methyl bromide at 25°C and 95% RH

Life stage	No.	Slope[a]	LC_{50} (mg/liter)	LC_{50} 95% CI	LC_{99} (mg/liter)	LC_{99} 95% CI	χ^2 (df)[b]
Egg	14,335	5.12 ± 1.32	7.10	0.02–14.01	20.18	3.33–27.10	88.8 (37)
Crawler	23,775	12.9 ± 0.45	25.10	23.88–26.2	38.02	35.49–41.91	309.3 (37)
Early nymph	4,650	16.2 ± 0.67	26.50	24.92–27.61	36.86	34.76–40.69	252.7 (22)
Late nymph	14,699	13.2 ± 0.28	25.02	24.02–25.92	37.57	35.25–41.15	1,846.3 (87)
Adult	463	17.1 ± 2.78	25.68	21.68–27.61	35.09	35.09–52.39	56.0 (17)

[a] Regression model significant ($P = 0.005$).
[b] Pearson chi-square statistic (degrees of freedom) significant ($P > 0.0001$).

Results

Susceptibility Tests. The nontransformed dose–response data for five life stages of *M. hirsutus* to 2-h methyl bromide fumigations fit a sigmoidal logistic function with $R^2 = 0.99$ ($F = 3922.8$; df = 2, 45; $P < 0.0001$) for eggs; $R^2 = 0.97$ ($F = 666.0$; df = 2, 45; $P < 0.0001$) for crawlers; $R^2 = 0.97$ ($F = 551.5$; df = 2, 32; $P < 0.0001$) early nymphs; $R^2 = 0.87$ ($F = 352.2$; df = 2, 109; $P < 0.0001$) for late nymphs; and $R^2 = 0.89$ ($F = 86.3$; df = 2, 21; $P < 0.0001$) for adults. The lowest dose producing complete control (100% mortality) was 19.8, 36.1, 34.0, 36.7, and 30.1 mg/liter for eggs, crawlers, early nymphs, late nymphs and adults, respectively.

Transformed dose–response data from probit regression analyses, reflecting the susceptibilities of the five life stages of *M. hirsutus* to methyl bromide in 2-h fumigations, are shown in Table 1. The egg stage was the most susceptible with an LC_{50} and LC_{99} of 7.1 and 20.2 mg/liter, respectively. With a slope of 5.1, the egg stage was the most heterogeneous in terms of susceptibility to methyl bromide. The four active life stages were significantly more tolerant than was the egg stage (≈3.5-fold and 1.8-fold at the LC_{50} and LC_{95}, respectively). LC_{50}s ranged from ≈25–26 mg/liter, and their LC_{99}s ranged from ≈35–38 mg/liter. No significant difference occurred in susceptibilities among the active life stages at either the LC_{50} or LC_{99} based on probit analyses. However, a paired comparison of mortalities between the nymphs fumigated at an average dose of 32.9 mg/liter showed that late nymphs were more tolerant (mean mortality ± SE = 68.5% ± 9.2) than early nymphs (95.2% ± 2.1) ($t = 3.49$, df = 4, $P = 0.025$). Slopes for the regression lines for the active life stages were steeper and more homogeneous in response than the slope for the egg stage (Table 1). Among these four stages, the regression lines fell into two groups with reference to their slopes. Crawlers and late nymphs had slopes of 12.9 and 13.2, respectively, whereas early nymphs and adults showed a more homogeneous response with slopes of 16.2 and 17.1, respectively. A test of parallelism showed that the four regression lines for the active life stages were not parallel (regression coefficient = 13.3 ± 0.215, df = 3, $P < 0.01$).

Confirmatory Tests. Mortality data for crawlers, late nymphs, and eggs of *M. hirsutus* fumigated 2 h at the target doses of 48 and 64 mg/liter are shown in Table 2. No individuals survived any treatment. Average doses of 48.8, 46.9, and 46.5 mg/liter produced 100% mortality of 10,751 crawlers, 2,732 nymphs, and 1,694 eggs, respectively. Average doses of 58.4, 58.4, and 60.1 mg/liter produced 100% mortality of 8,336 crawlers, 2,514 nymphs, and 1,604 eggs, respectively.

Discussion

In our laboratory fumigations, methyl bromide was effective in controlling *M. hirsutus.* Even though the active life stages were considerably more tolerant to the fumigant than was the egg, complete control of all tested life stages was achieved at a dose of 48 mg/liter and above (Table 2). Regression analyses showed that all active life stages were equally tolerant to methyl

Table 2. Mortality data for eggs, late nymphs, and crawlers of *M. hirsutus* from confirmatory tests of 2-h fumigations with methyl bromide according to the USDA–APHIS Treatment Schedule T-104-a-2 for mealybugs at 25°C, 95% RH

Life stage	Target dose, 48 mg/liter				Target dose, 64 mg/liter			
	Actual dose	Range	No.	Mortality, %	Actual dose	Range	No.	Mortality, %
Eggs	0[a]		311	22	0[a]		539	11
	46.5[b]	40.9–49.1	1,694	100	60.1[b]	57.1–64.0	1,604	100
Crawlers	0[a]		2,421	5	0[a]		1,493	5
	48.8[b]	43.9–51.0	10,751	100	58.3[b]	52.8–62.5	8,336	100
Late nymphs	0[c]		363	16	0[c]		603	8
	46.9[d]	40.9–49.6	2,732	100	58.3[d]	52.8–64.0	2,514	100

[a] Data pooled from two tests, one replicate dose per test.
[b] Data pooled from two tests, five replicate doses per test.
[c] Data from one test, one replicate dose per test.
[d] Data from one test, five replicate doses per test.

bromide (Table 1). The slopes of these regression lines were unique, but there was overlap of the confidence intervals such that the LC_{50} and LC_{99} values were not significantly different. However, the paired *t*-test directly comparing early and late nymphs showed that late nymphs were more tolerant than early nymphs.

Pilgrim (1998) demonstrated that a methyl bromide dose of 24 mg/liter at an unspecified temperature was sufficient to control all life stages of *M. hirsutus* in Grenada; this dose is recommended for control of *M. hirsutus* in the Caribbean. LC_{99}s for our Hawaiian strain (Table 1) were higher (35–38 mg/liter) than the dose (24 mg/liter) recommended for complete control of the Caribbean strain (Pilgrim 1998). The APHIS treatment schedule for mealybugs recommends a dose of 48 mg/liter between 21 and 26°C (USDA 1998). Thus, based on our toxicity data, the treatment schedule will provide quarantine security against *M. hirsutus.*

The Montreal Protocol mandates the phase out of methyl bromide by 2005 (UNEP 1992). However, to avoid nontariff trade barriers and because alternative technologies were not yet widespread and available, the Montreal Protocol exempted quarantine and preshipment fumigations. Nevertheless, many reasons exist to rescind this exemption (Batchelor 2001). Until a suitable replacement treatment is developed, methyl bromide should be available for mealybug fumigations.

Currently, introduced populations of *M. hirsutus* in Imperial County, CA, exist in urban areas only and have not migrated into commercial agricultural areas. A biological control program (Meyerdirk et al. 1998) was instituted two years ago, and rigorous inspections have failed to detect this pest in commercial agricultural crops nearby. Because of the success of the biological control program and the inspections, embargoes imposed earlier have been lifted, although the Mexico and Central America quarantines remain in place. The biological control program has significantly reduced *M. hirsutus* populations by 96% in the infested areas of Imperial County, but it is not expected to eradicate them (Meyerdirk 2001). Based on a climax model, it is estimated that this mealybug pest could establish in the 17 most southern states (D. Meyerdirk, USDA, APHIS, Riverdale, MD, personal communication). With *M. hirsutus* populations juxtaposed to agricultural areas in California, and with its continued presence in the Caribbean and Northern Baja, *M. hirsutus* probably will migrate to these additional areas in the United States, disrupting agricultural trade and warranting quarantine fumigations.

Any treatment that is successful in controlling pests in agricultural commodities should have little or no deleterious effects on the quality of the commodity being treated (Aung 1998). However, many commodities are susceptible to quality deterioration following fumigation with methyl bromide (McDonald and Miller 1994). Even at the relatively low dose rate of 24 mg/liter recommended for control of *M. hirsutus* in the Caribbean area, methyl bromide can be phytotoxic to a variety of fresh produce (Pilgrim 1998). However, some commodities (i.e., table grapes) are quite tolerant to large doses of methyl bromide (Smilanick et al. 2000). Thus, before any treatment schedule can be considered effective, it must be determined whether that treatment reduces the value of the commodity.

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